昆 虫 学 报 *Acta Entomologica Sinica*, May 2016, 59(5): 523 - 529　　doi:10.16380/j.kcxb.2016.05.006

Morphology and ultrastructure of the spermatheca of *Pararcyptera microptera meridionalis* (Orthoptera: Arcypterinae)

LI Ke*, YANG Yu-Jing, MAO Lei, ZHANG Jin-Hui

(College of Life Sciences, Shanxi Normal University, Linfen, Shanxi 041004, China)

Abstract:【Aim】To ascertain the morphology, organization structure and ultrastructure of the spermatheca in females of *Pararcyptera microptera meridionalis* (Orthoptera: Arcypterinae) for providing a better understanding of the functions of insect spermatheca.【Methods】The morphology, organization structure and ultrastructure of spermatheca of mated female adults of *P. m. meridionalis* were examined by means of the light and transmission electron microscopy.【Results】The spermatheca is composed of a seminal receptacle, mainly serving for storage of spermatozoa, and a long spermathecal duct. Histologically, both parts have an outer muscle coat followed by a basal lamina, epithelium, and cuticular intima. The epithelium is made up of three cell types, *i. e.*, epithelial cells, gland cells and duct cells. Each gland cell has a reservoir lined with microvilli. The secretions of gland cell are released into the reservoir through which it reaches the spermathecal lumen via the secretory duct formed by the duct cell.【Conclusion】Regional differences in the ultrastructure of the cuticular intima and the reservoir in gland cell imply that the seminal receptacle and the spermathecal duct carry out different functions. The ultrastructural features revealed that epithelial cells are involved in the function of support, secretion and absorption.

Key words: *Pararcyptera microptera meridionalis*; spermatheca; ultrastructure; epithelial cells; gland cells; duct cells

1 INTRODUCTION

The spermatheca is an organ of ectodermal origin in the reproductive system of female insects. It serves as a repository for spermatozoa during copulation. Within the spermatheca, the spermatozoa remain viable for a long time under the influence of glandular secretions. They are thereafter delivered to the site of fertilization during oviposition by means of the contractions of the spermathecal muscle (Lange and Silva, 2007).

The spermatheca shows notable differences in shape, number, and structure among various species. In general, insects have one spermatheca, which is composed of a seminal receptacle and a spermathecal duct. The seminal receptacle exhibits all kinds of shapes: piriform in *Platycleis grisea* (Brundo *et al.*, 2011), sac-shaped in *Platycleis intermedia* (Brundo *et al.*, 2011), spherical in *Teleogryllus commodus* (Sturm, 2008), flattened tubular in *Oxya chinesis* (Dou and Xi, 2002), and ring-shaped in *Gryon pennsylvanicum* (Paoli *et al.*, 2014). Sometimes, there are two spermathecae, as in *Blaps* (Coleoptera) and *Phlebotomus* (Diptera) (Chapman, 1998). Most of higher flies have three spermathecae (Pascini *et al.*, 2012).

The site where the spermathecal duct opens into the female gonoduct is likewise multivariate among different species. One arrangement is the spermatheca opening into the genital chamber independently of the common oviduct (orthopteran). Another arrangement is the genital chamber becoming tubular, the spermatheca opening internal within the common oviduct (lepidopteran) (Chapman, 1998).

Numerous studies have been made concerning the morphology and structure of the spermatheca: *Melanoplus sanguinipes* (Ahmed and Gillott, 1982), *Gesonula punctifrons* (Paland and Ghosh, 1982), *Locusta migratoria* (Lay *et al.*, 1999), *T. commodus* (Sturm, 2008), and some Tettigoniidae (Brundo *et al.*, 2011) among the Orthoptera. However, almost nothing is known about Orthoptera Arcypterinae. Our investigations centered on the morphology and ultrastructure of the spermatheca in *P. m. meridionalis* (Orthoptera: Arcypterinae) in order to complement older data, and to provide a better understanding of the functions of insect spermatheca.

基金项目：山西师范大学博士启动金(833202)；山西师范大学生创新性实验项目(SD2013CXCY-74)

作者简介：李柯，男，1974 年 9 月生，山西晋城人，博士，副教授，研究方向为昆虫行为生理学，E-mail：like3515@126.com

* 通讯作者 Corresponding author, E-mail: like3515@126.com

收稿日期 Received: 2016-02-14; 接受日期 Accepted: 2016-03-22

2 MATERIALS AND METHODS

Healthy insects were collected from Huo Mountain in Linfen, Shanxi province, China. They were fed in the laboratory. Mated female adults were then dissected in a saline solution, and spermathecal morphology was observed under a stereo-microscope. For histological investigations, the spermathecae were fixed in Bouin's liquid for 4 h at 4℃, dehydrated in an alcohol series for 10 min in each bath, and embedded in paraffin. The 7 μm sections were stained with Haematoxylin-Eosin.

For the transmission electron microscopy (TEM) investigations, spermathecae were fixed in 2.5% glutaraldehyde for 4 h at 4℃. After being washed with 0.1 mol/L phosphate buffer saline (PBS) (pH 7.4) three times, samples were post-fixed in 1% osmium tetroxide with a phosphate buffer for 2 h, and washed in 0.1 mol/L PBS once more. The preparations were subsequently dehydrated in a standard ethanol series, and embedded in Epon-812. Ultrathin sections (60 nm) were cut with a glass knife, collected on copper, stained with uranyl acetate and lead citrate, and then photographed with an H-7650 transmission electron microscope.

3 RESULTS

3.1 Morphology and histology of the spermatheca of *P. m. meridionalis*

The spermatheca of orthopteran *P. m. meridionalis* consists of a distal bean-shaped, sclerotized section called the seminal receptacle (*ca.* 3 mm in length; 1 mm in width at its widest point) and a tightly coiled spermathecal duct (*ca.* 5 cm in spread length) with a almost uniform diameter (*ca.* 0.2 mm) throughout its length except for a thick basal portion (Fig. 1). The distal duct arises from the base of bean and shapes into a U-shape together with the bean (Fig. 1: B), and then twists through several loops before finally joining into the genital chamber (Fig. 1: A). The dense secretions and sperm mass in the lumen of both the seminal receptacle and the spermathecal duct were found beneath the light microscope (Fig. 2: A).

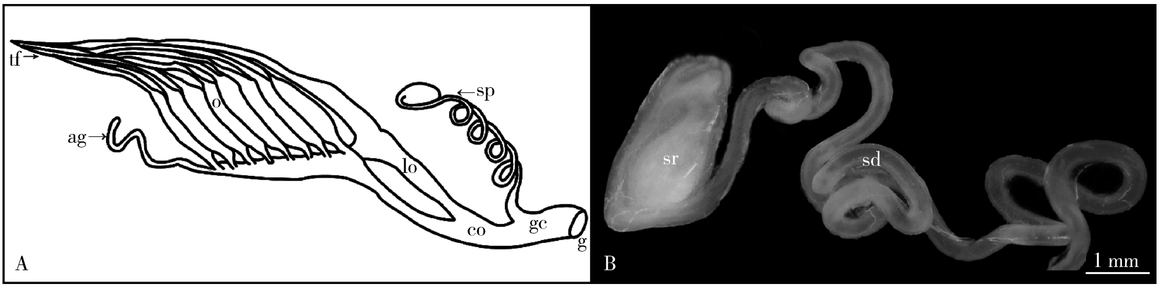

Fig. 1 General view of the spermatheca of *Pararcyptera microptera meridionalis*

A: Diagram of the female reproductive systems; B: Overall view and shape of the seminal receptacle and part spermathecal duct which is extended under stereo microscope. ag: Accessory gland; co: Common oviduct; g: Gonopore; gc: Genital chamber; lo: Lateral oviduct; o: Ovariole; sp: Spermatheca; tf: Terminal filament; sr: Seminal receptacle; sd: Spermathecal duct.

The general organization of the wall of seminal receptacle in *P. m. meridionalis* is similar in the various tracts of the spermathecal duct (Fig. 2). Histological sections indicate that the wall of the spermatheca consists of the cuticular intima, epithelial cells, gland cells, duct cells, basal lamina, and muscle (Fig. 2). The seminal receptacle appears as dilated sac, and its lumen is covered by a continuous cuticular lining (Fig. 2: A). Outside the cuticular intima is a pseudo-stratified epithelium that includes epithelial cells, gland cells, and duct cells (Fig. 2: B and C). The basal lamina is itself coated with the epithelium, and divides the epithelium from muscle. The muscles of spermatheca are composed of transverse and longitudinal muscle fibers.

3.2 Ultrastructure of the spermatheca of *P. m. meridionalis*

The cuticular intima of *P. m. meridionalis* shows a typical structure made up of a thick endocuticle, an exocuticle with homogeneous aspects, and a thin epicuticle. The thin epicuticle has an intensely osmiophilic layer that delimites the inner lumen (Fig. 3). More strikingly, distinct acanthae appear on the inner side of the intima surface of the seminal receptacle (Fig. 3: A and C) and they face the seminal receptacle lumen. It is smooth on the corresponding side of the spermathecal duct (Fig. 3: B). Some acanthae and the viscous sperm mass stick together in the seminal receptacle (Fig. 3: C).

The three aforementioned cell types were identified along the spermathecal epithelium. They are tightly packed (Fig. 4: A). Epithelial cells and duct cells are arranged in the apical part of the epithelium, and their lateral continuous plasma membranes form septate junctions with deep interdigitations (Fig. 4: B).

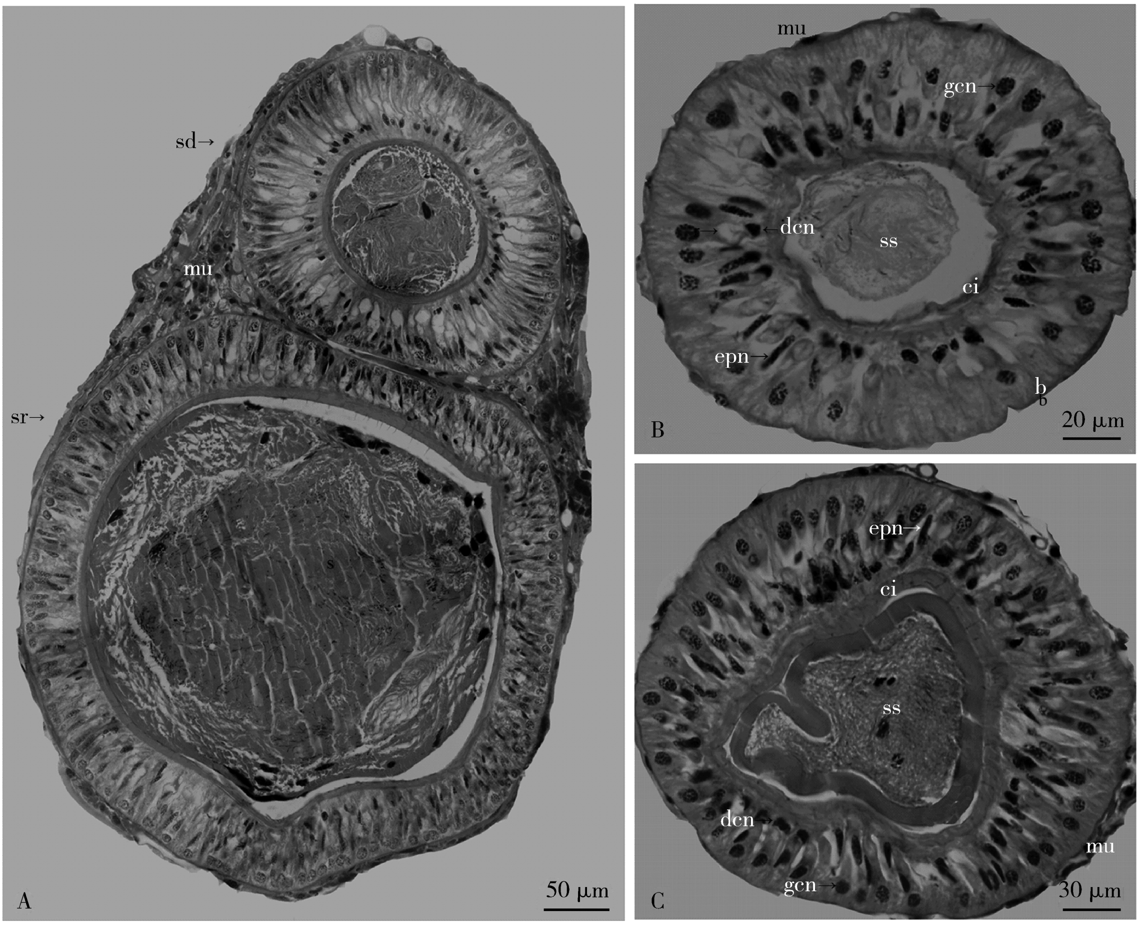

Fig. 2　Histological sections of the spermatheca of *Pararcyptera microptera meridionalis*

A: Cross-section of the seminal receptacle and the distal spermathecal duct; B: Transection of the middle part of the spermathecal duct; C: Cross-section of the basal spermathecal duct. sr: Seminal receptacle; sd: Spermathecal duct; ss: Sperm and secretions; mu: Muscle; ci: Cuticular intima; epn: Epithilial cell nucleus; gcn: Gland cell nucleus; dcn: Duct cell nucleus; r: Reservoir; b: Basal lamina.

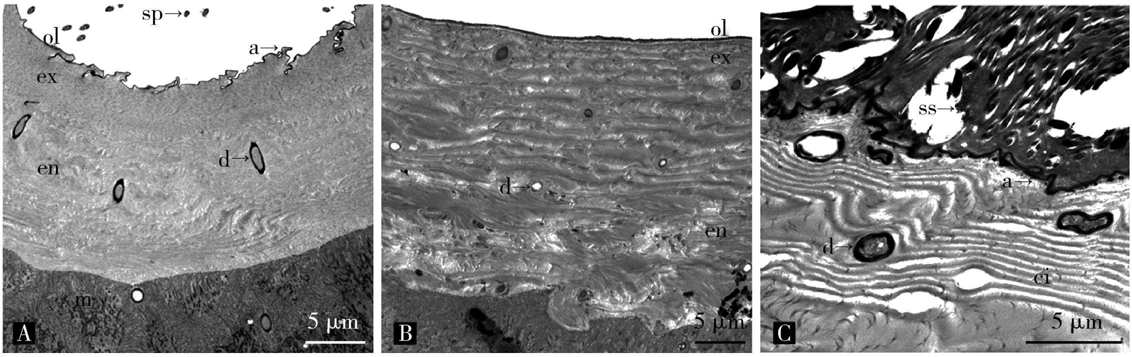

Fig. 3　Cuticular intima of the spermtheca of *Pararcyptera microptera meridionalis*

A: Cuticular intima of the seminal receptacle; B: Cuticular intima of the spermathecal duct; C: Acanthae in the cuticular intima and the viscous sperm mass sticks together in the seminal receptacle. a: Acanthae; d: Secretory duct; sp: Sperm; ol: Osmiophilic layer; ex: Exocuticle; en: Endocuticle; m: Mitochondria; ss: Sperm mass and secretions; ci: Cuticular intima.

Epithelial cells are characterized by slender nuclei with heterochromatin, and have relatively less cytoplasm and organelles (Fig. 4: A). In apical cytoplasm, densely lined microvilli (microvillous border) are found towards the cuticular intima (Fig. 4: B).

Duct cells are specialized for producing secretory ducts that connect reservoirs of gland cells with the spermathecal lumen (Fig. 5: A). One end of the secretory duct that projects into the secretory reservoir is closed off by an end apparatus (Fig. 5: A and B). The other end that traverses the cuticular intima opens into the inner surface of the cuticular intima in both the seminal receptacle and the spermathecal duct (Fig. 3). Tubules with bilayer walls are filled with various electron-dense materials (Fig. 3). Multiple sections

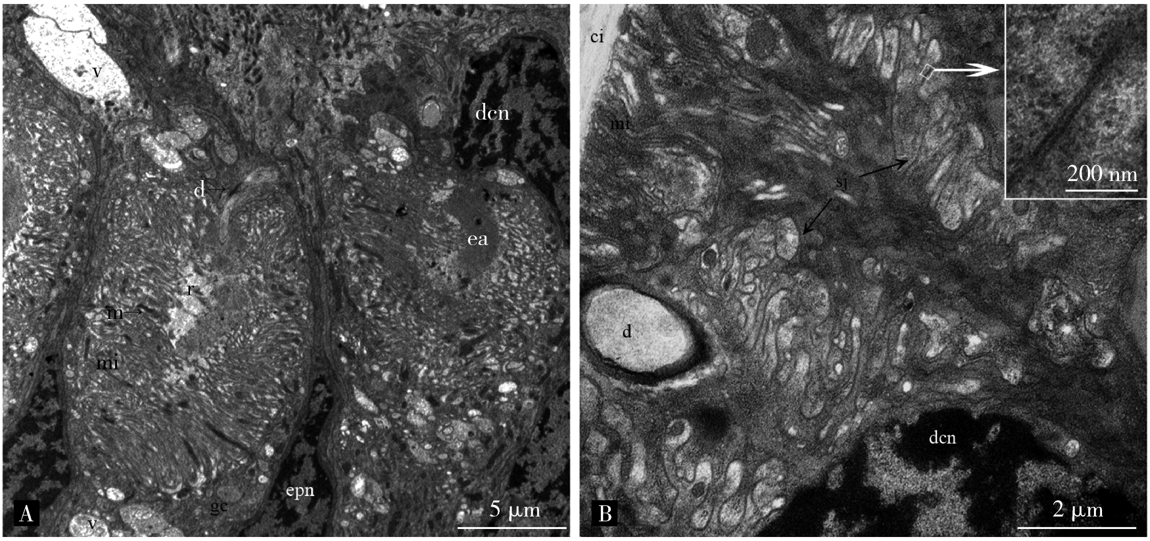

Fig. 4 Relationship between three cell types in spermthecal epithelium of *Pararcyptera microptera meridionalis*
A: Epithelial cells, gland cells and duct cells are tightly packed; B: Contiguous cytoplasmic memebranes form septate junctions (black arrows) with deep interdigitation. The inset shows the dilation of the septate junctions in the rectangle (white arrow). v: Vesicle; d: Secretory duct; r: Secretory reservoir; m: Mitochondria; mi: Microvilli; gc: Gland cell; epn: Epithelial cell nucleus; dcn: Duct cell nucleus; ea: End apparatus; ci: Cuticular intima; sj: Septate junctions.

can be seen through the secretory duct within the same cell. Irregularly shaped nuclei are usually closed to the secretory reservoir of the gland cell (Fig. 4: A and Fig. 5: A). Cytoplasm of the duct cell is more electron-dense than that of the gland cell.

Gland cells manifest cellular structures of classic secretory cells: mitochondria, ribosomes, large flattened sacs of rough endoplasmic reticulum (RER), and vesicles of various sizes (Fig. 5: C – E). Each cell contains a large secretory reservoir that almost occupies the entire apical part of gland cell (Fig. 4: A and Fig. 5: C). Vesicles that vary in size are abundant in the basal cytoplasm and around the reservoir (Fig. 5: C and D). Moreover, some electron-lucent vesicles touch the membrane of the reservoir, and some are directly released into the reservoir (Fig. 5: D). Secretions are sometimes observed within the reservoir, acting as a temporary storage-site (Fig. 5: D). Most notably, there are no mitochondria around the sparse microvilli that line the reservoir in the seminal receptacle (Fig. 5: C and D). In contrast, the reservoir in the spermathecal duct shows a small amount of mitochondria and dense microvilli (Fig. 4: A and Fig. 5: A). The nucleus of gland cells is mainly located in the basal position, between the secretory reservoir and the basal lamina. The RER is present throughout all regions of the epithelium and is especially evident in the perinuclear region of the gland cells (Fig. 5: E).

Moreover, mitochondria are concentrated in the apical part of epithelial cells and in the basal labyrinth areas (Fig. 6). All cell types lie on a fibrillar basal lamina. Their basal plasmic membrane together form a basal labyrinth system by means of developing a highly infolded membrane (Fig. 6: B). The basal lamina runs a straight course and is always surrounded by connective muscle theca, which is formed by a thick layer of longitudinal muscles and transverse muscles.

4 DISCUSSION

The *P. m. meridionalis* spermatheca is composed of a single seminal receptacle and a spermathecal duct that opens into the genital chamber. This is similar to other insects of Orthoptera: *G. punctifrons* (Paland and Ghosh, 1982), *M. sanguinipes* (Ahmed and Gillott, 1982), *L. migratoria* (Lay *et al.*, 1999), and *Tylopsis liliifolia* (Viscuso *et al.*, 2015). In terms of histology, our results show that the general organization of the spermahteca (in order of internal outwards: cuticular intima, epithlium, basal lamina, and muscle) is fairly uniform in most species so far examined.

There are two types of epithelium in the spermathecae which have been investigated. The first type itself consists of two cell types: epithelial cells and gland cells. The former cell is responsible for the formation of the cuticular intima and the duct, as seen in *G. punctifrons* (Paland and Ghosh, 1982), *L. migratoria* (Lay *et al.*, 1999), *Schistocerca Americana* and *Dissosteira Carolina* (Gardner, 2004), *P. intermedia* (Brundo *et al.*, 2011). The second type of epithelium includes a third cell type, duct cells. They specifically form the secretory duct. This is seen in *Oedaleus infernalis* Saussure (He *et al.*, 2004), *Zorotypus caudelli* Karny (Dallai *et al.*, 2012) and *P. m. meridionalis* (our present study). Thus, it is suggested that insects with all three cell types may be more specialized than those with only the former two.

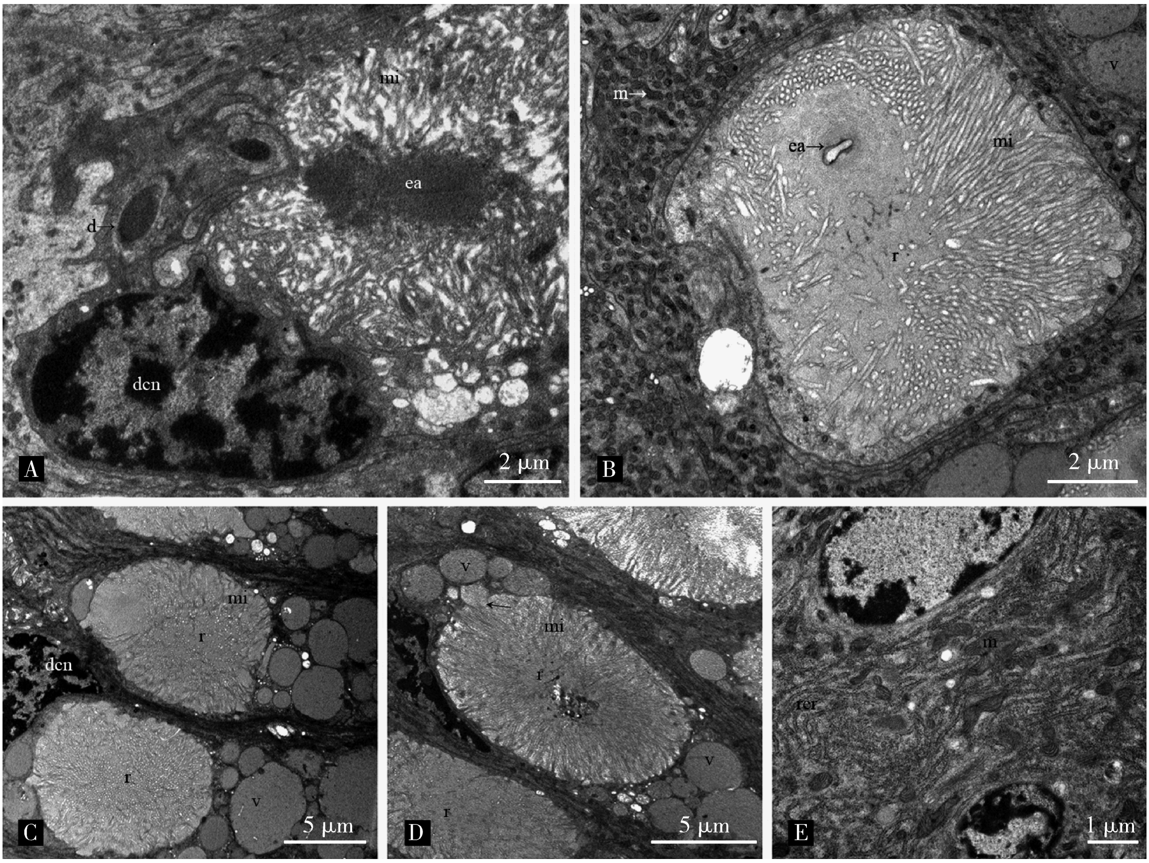

Fig. 5 TEM micrographs of the spermthecal duct cell and gland cell in *Pararcyptera microptera meridionalis*

A: Duct cell with electron-dense cytoplasm and end apparatus in reservoir are observed; B: Transection of secretory reservoir in gland cell, the end apparatus can be observed; C, D: Gland cells in the seminal receptacle. C: A large secretory reservoir almost occupys the apical part of the gland cell. Basal is to the right; D: Some vesicles around the reservoir seem to open into the reservoir (arrow); E: Rough endoplasmic reticulum and mitochondria is evident in perinuclear region of the gland cells. mi: Microvilli; ea: End apparatus; dcn: Duct cell nucleus; d: Secretory duct; m: Mitochondria; r: Secretory reservoir; v: Vesicles; rer: Rough endoplasmic reticulum.

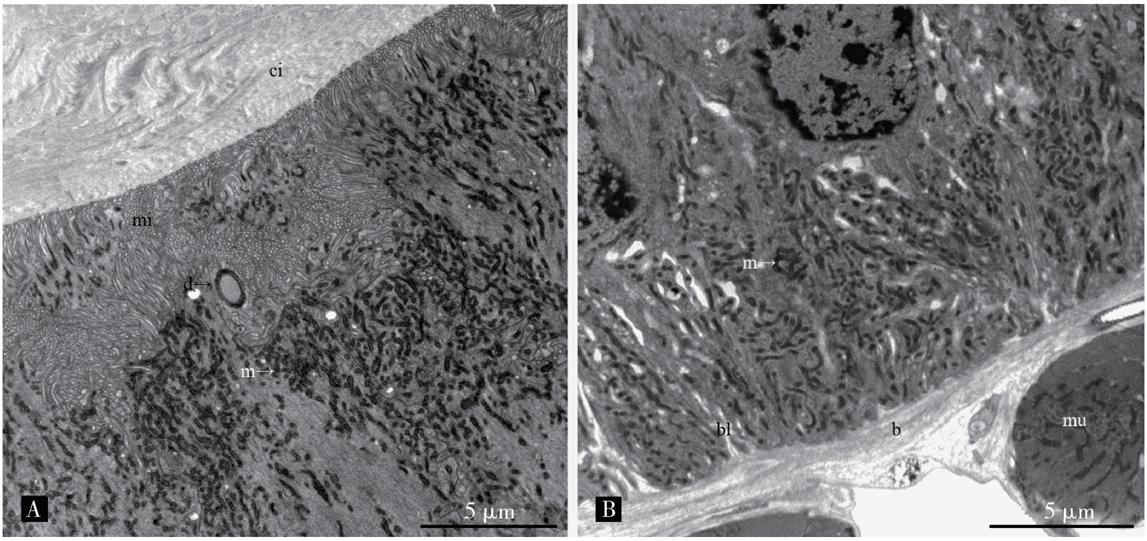

Fig. 6 Apical and basal regions of the spermthecal epithelium in *Pararcyptera microptera meridionalis*

A: Densely lined microvilli towards the cuticular intima and numerous mitochondria are found in apical cytoplasm of the epithelial cell; B: Basal plasmic membrane highly infold and form a basal labyrinth system. Large amounts of mitochondria are also concentrated in the basal areas. ci: Cuticular intima; mi: Microvilli; d: Secretory duct; m: Mitochondria; b: Basal lamina; bl: Basal labyrinth; mu: Muscle.

Further research using other techniques is needed to verify this viewpoint.

Previous literature demonstrates that the acanthae on the surface of the cuticular intima exist in some insects. The distribution highlights regional difference. For example, in *O. infernalis* (He *et al.*, 2004) and

P. m. meridionalis (present study), acanthae are observed only on the surface of cuticular intima in the seminal receptacle (Fig. 3: A and C). In contrast with *M. sanguinipes* (Ahmmed and Gillott, 1982) and *L. migratoria* (Lay *et al.*, 1999), acanthae are on the transition region between the seminal receptacle and the spermathecal duct. In addition to this, in the spermatheca of *Dichromorpha viridis* and *Chortophaga viridifasciata* (Johnson, 1998) the acanthae are in both the seminal receptacle and the transition region. However, in *S. americana* and *D. carolina* (Gardner, 2004), no acanthae are on the inner surfaces of the spermatheca. Some researchers think that acanthae permit the liquid which the viscous sperm mass is pressed into the receptacle, and maintain at a distance from the wall (Lay *et al.*, 1999). But others argue that the functions are merely mechanical as they are without sockets and not movable (Richards and Richards, 1979). Moreover, they argue that the acanthae prevent removal of the sperm bundle during copulation, but allow individual sperm to be displaced (Johnson, 1998). Our results show that the acanthae and the viscous sperm mass stick together (Fig. 3: C). Thus we speculate that sperms fully contact the lumenal secretions inside the seminal receptacle in order to keep the sperm alive. Further investigation is needed to verify the role of acanthae inside the spermatheca.

From TEM observations, mitochondria, dominant cytoplasmatic organelles, and the rough endoplasmic reticulum are shown to be widespread in the cytoplasm of epithelial cells and gland cells. Furthermore, it reveals that these cells present well-developed synthesis apparatus in which typical cells are involved in secretory activities. Large amounts of mitochondria are especially concentrated in the apical and in the basal labyrinth areas (Fig. 6). Therefore, it is clear that the spermathecal epithelium provides a suitable osmotic environment by means of transporting substances.

Especially striking are regional differences about the secretory reservoir in the different regions of the spermatheca. There are sparse microvilli and relatively homogeneous vesicles in the seminal receptacle (Fig. 5: C and D) while there are densely microvilli and heterogeneous vesicles in the speramthecal duct (Fig. 4: A and Fig. 5: A). Moreover, in the seminal receptacle, some of vesicles around the reservoir seem to release the substance into the reservoir (Fig. 5: D). We speculate these gland cells may have merocrine secretion. This phenomenon is also observed in *Tessellana tessellata* (Brundo *et al.*, 2011). However, in the speramthecal duct, there are mitochondria among the microvilli. We may therefore presume that substances can be released or absorbed via the microvilli by means of active transport processes. These results indicate that gland cells have different functions in these two areas.

One last interesting observation concerns the multiple sections seen within the same duct cell (Fig. 5: A). This is most readily explained by a highly sinuous organization within the duct cell. Furthermore, there are two possible reasons for various electron-dense materials in the secretory duct (Fig. 3). The first is that the secretions from gland cells may have had biochemical changes inside the duct. The second is that different kinds of secretions directly derive from gland cell. The components of these secretions are unknown.

In light of these results, we find that the morphology and structure of *P. m. meridionalis* spermatheca is similar to other insects in Orthoptera. The spermathecal epithelium includes three cell types, *i. e.*, epithelial cells, gland cells and duct cells. The structural differences of cuticular intima and the secretory reservoir imply that the seminal receptacle and the spermathecal duct have different functional roles.

References

Ahmed I, Gillott C, 1982. The spermatheca of *Melanoplus sanguinipes* (Fabr.). II. Ultrastructure. *Int. J. Invertebr. Reprod.*, 4(5): 297-309.

Brundo MV, Longo G, Sottile L, Trovato M, Vitale D, Viscuso R, 2011. Morphological and ultrastructural organization of the spermatheca of some Tettigoniidae (Insecta, Orthoptera). *Ital. J. Zool.*, 78(1): 53-62.

Chapman RF, 1998. The Insects: Structure and Function. 4th ed. Cambridge University Press, Cambridge. 297.

Dallai R, Mercati D, Gottardo M, Machida R, Mashimo Y, Beutel RG, 2012. The fine structure of the female reproductive system of *Zorotypus caudelli* Karny (Zoraptera). *Arthropod Struct. Dev.*, 41(1): 51-63.

Dou XM, Xi GS, 2002. Observations on the microstructure and ultrastructure of spermatheca in *Oxya chinesis*. *Chin. J. Zool.*, 37(6): 5-7. [窦向梅, 奚耕思, 2002. 中华稻蝗受精囊的显微与超微结构. 动物学杂志, 37(6): 5-7]

Gardner GE, 2004. Morphological and Histological Aspects of the Spermatheca as They Relate to Sperm Organization in the Grasshopper Species *Schistocerca americana* and *Dissosteira carolina* (Orthoptera: Acrididae). MSc Thesis, Faculty of North Caroliina State University, Raleigh. Available at: http://repository.lib.ncsu.edu/ir/handle/1840.16/2927.

He JP, Xi GS, Ren YH, 2004. Ultrastructure of spermatheca in the grasshopper *Oedaleus infernalis* Saussure (Orthoptera: Acrididae). *Acta Entomol. Sin.*, 47(6): 725-731. [何建平, 奚耕思, 任耀辉, 2004. 黄胫小车蝗受精囊的亚显微结构. 昆虫学报, 47(6): 725-731]

Johnson JA, 1998. A Histological Comparative Study on Sperm Competition inside the Spermathecae in the Grasshopper Species, *Dichromorpha viridis* and *Chortophaga viridifasciata* (Orthoptera: Acrididae). MSc Thesis, Faculty of North Caroliina State University, Raleigh. Available at: http://www.lib.ncsu.edu/resolver/1840.16/1487.

Lange AB, Silva RD, 2007. Neural and hormonal control of muscularactivity of the spermatheca in the locust, *Locusta migratoria*. *Peptides*, 28(1): 174–184.

Lay M, Zissler D, Hartmann R, 1999. Ultrastructural and functional aspects of the spermatheca of the African migratory locust *Locusta migratoria migratorioides* (Reiche and Fairmaire) (Orthoptera: Acrididae). *Int. J. Insect Morphol. Embryol.*, 28(99): 349–361.

Paland SG, Ghosh D, 1982. Electron microscopic study of the spermatheca of *Gesonula punctifrons* (Acrididae: Orthoptera). *Proc. Indian Acad. Sci. Anim. Sci.*, 91(2): 99–112.

Paoli F, Gottardo M, Marchini D, Dallai R, Roversi PF, 2014. Ultrastructure of the female reproductive apparatus of the egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae). *Micron*, 61(8): 28–39.

Pascini TV, Ramalho-Ortigão M, Martins GF, 2012. Morphological and morphometrical assessment of spermathecae of *Aedes aegypti* females. *Mem. Inst. Oswaldo Cruz*, 107(6): 705–712.

Richards AG, Richards PA, 1979. The cuticular protuberances of insects. *Int. J. Insect Morphol. Embryol.*, 8(3–4): 143–157.

Sturm R, 2008. Morphology and histology of the ductus receptaculi and accessory glands in the reproductive tract of the female cricket, *Teleogryllus commodus*. *J. Insect Sci.*, 8(1): 53–56.

Viscuso R, Vitale DGM, 2015. Spermatodesm reorganization in the spermatophore and in the spermatheca of the bushcricket *Tylopsis liliifolia* (Fabricius) (Orthoptera, Tettigoniidae). *Arthropod Struct. Dev.*, 44(3): 243–252.

宽翅曲背蝗受精囊形态与超微结构观察

李柯*，杨宇菁，毛蕾，张晋辉

（山西师范大学生命科学学院，山西临汾 041004）

摘要：【目的】明确宽翅曲背蝗 *Pararcyptera microptera meridionalis* 雌虫受精囊的形态、组织结构与超微结构，为更好地认识昆虫受精囊的功能提供依据。【方法】本研究以宽翅曲背蝗已交配雌成虫为实验材料，利用光学显微镜和透射电子显微镜观察其受精囊的形态、组织结构和超微结构。【结果】宽翅曲背蝗受精囊由一个端囊和一条长的受精囊管组成，端囊用于储存精子。端囊和受精囊管有相似的组织学结构，由外到内依次为肌肉层、基膜、上皮层及表皮内膜。上皮层含上皮细胞、腺细胞和导管细胞3种细胞类型。腺细胞具有一个被有微绒毛的细胞外腔。腺细胞的分泌物经细胞外腔通过分泌导管进入到受精囊腔。分泌导管由导管细胞形成。【结论】在宽翅曲背蝗受精囊的端囊和受精囊管上，内膜和腺细胞的细胞外腔结构均存在差异，由此推测，端囊和受精囊管的功能存在一定差异。上皮细胞的超微结构特点显示上皮细胞具有支持、分泌和吸收的功能。

关键词：宽翅曲背蝗；受精囊；超微结构；上皮细胞；腺细胞；导管细胞

中图分类号：Q964　　**文献标识码：**A　　**文章编号：**0454-6296(2016)05-523-07

（责任编辑：赵利辉）